REMARKS

Claims 1-4 and 8-9 are currently pending in the application. The specification and claims 4 and 9 have been amended. The amendments are discussed in the relevant sections below, and find support in the specification as originally filed and in the documents from which priority is claimed. No new matter is added.

The rejection of claim 8 under 35 U.S.C. § 112, first paragraph, claims 1-2 under 35 U.S.C. § 112, first paragraph, claims 1-4 and 8-9 under 35 U.S.C. § 102(b) and claims 1-4 and 8-9 on double patenting grounds are not addressed here. Instead, Applicant has filed herewith a Notice of Appeal. These rejections will be addressed at a later date.

The following amendments address various formalities regarding the specification, and do not introduce any new requirements for additional search or consideration on the part of the Examiner. Their entry is therefore respectfully requested.

Sequence Compliance

The Examiner states that SEQ ID NO:10 of the sequence Listing does not correspond to the sequence shown in Fig. 18B.

In U.S. App. No. 09/335,225, Applicant has been requested to review all sequences disclosed in the specification in this and all related cases. This Applicant has done. In view of the disclosure of sequences in U.S. App. No. 60/089,689 (filed June 17, 1998), 60/126,175 (filed March 25, 1999), U.S. App. No. 09/335,224 (filed June 17, 1999), and in the present case as originally filed, Applicant submits herewith (1) an amendment to the specification at page 44, lines 3-5, (2) substitute Figs. 18A and 18B, and (3) a substitute Sequence Listing. The individual amendments made are itemized and discussed below. Applicant submits that the amendments contain no new matter, and are described in the application and priority documents as originally filed. Applicant respectfully requests entry of the amendments into the application.

Tumstatin Amino Acid Sequence (SEQ ID NO:10)

Applicant has found that the amino acid sequence for full-length Tumstatin was first presented in U.S. App. No. 60/089,689, filed June 17, 1998, as an amino acid sequence of 244

amino acid residues. Figs. 9 and 10of U.S. App. No. 60/089,689, provided herewith as Exhibit A, shows nucleotide and amino sequences of the HGA3.3 exons of human α3(IV) cDNA. The description for Fig. 9, on page 4, lines 27-33, of U.S. App. No. 60/089,689, states that "Tumstatin begins at about residue 42 ("*"), and ends at about residue 285 ("**")." In Fig. 9, the amino acid sequence demarcated by "*" and "**" begins with "GLKG" and ends with "KKRH."

In U.S. App. No. 09/335,224, the sequences depicted in Fig. 9 of U.S. App. No. 60/089,689 were split into two figures, depicting the nucleic acid sequence (Fig. 16A, SEQ ID NO:9) and the amino acid sequence (Fig. 16B, SEQ ID NO:10) of Tumstatin. These drawings were presented in the present case as Figs. 18A and 18B.

In U.S. App. No. 09/335,224, Fig. 16B as originally filed did not begin with the sequence at the "*" symbol of Fig. 9 of U.S. App. No. 60/089,689, but instead began with the first complete line of text in which the Tumstatin protein sequence began in Fig. 9 of that application, with the amino acid residues making up the Tumstatin protein sequence indicated by a handwritten circle. The text in the specification at page 17, lines 1-2 us U.S. App. No. 09/335,224 referred to the entire sequence in the drawing, in an error made with no deceptive intent. During the preparation of the formal drawings, a clerical error resulted in this handwritten circle being ignored, resulting in the inadvertent inclusion of the amino acid residue "P" at the beginning of the Tumstatin protein. Formal Fig. 16B, filed on November 22, 1999 therefore depicted the beginning of the Tumstatin protein sequence as "PGLKG" rather than "GLKG", resulting in a protein of 245 amino acids, rather than 244. The Sequence Listing submitted on November 22, 1999 likewise included the same error.

A substitute Fig. 16A, 16B and substitute Sequence Listing have been filed in U.S. App. No. 09/335,224.

Submitted herewith is an amendment to the specification at page 44, lines 3-5, and a substitute formal Fig. 18B, with the Tumstatin protein sequence depicted as starting with the residues "GLKG" rather than "PGLKG". The substitute Fig. 18B is therefore in accordance with the depiction of this sequence in Fig. 9 of U.S. App. No. 60/089,689, and substitute Fig. 16B in U.S. App. No. 09/335,224. The Sequence Listing submitted on February 8, 2001 correctly

presented SEQ ID NO:10 as being 244 amino acids long. The substitute Sequence Listing filed herewith also lists this protein as 244 amino acids in length.

Tumstatin Nucleic Acid Sequence (SEQ ID NO:9)

The inadvertent error discussed above was also made with the Tumstatin nucleic acid sequence (SEQ ID NO:9) which is depicted in Fig. 18A. Originally-filed Fig. 18A states at the bottom, "pET22bα3(IV) NC1 4-735", indicating that it was nucleotides 4-735 that were cloned into the pET22b vector as described in Example 23.

The various appearances of the Tumstatin nucleic acid sequence (SEQ ID NO:9) are listed below, with the changes over U.S. App. No. 60/089,689 indicated by boxes:

U.S. App. No. 60/089,689:	
(1) Fig. 9:	5′-ggt-ttg-aaa-gga-aaa-cgt-gga-...-3′
U.S. App. No. 60/126,175:	
(2) Fig. 9:	5′-ggt-ttg-aaa-gga-aaa-cgt-gga-...-3′
U.S. App. No. 09/335,224 (as originally filed):	
(3) Fig. 16A:	5′-ggt-ttg-aaa-gga-aaa-cgt-gga-...-3′
U.S. App. No. 09/335,224: (as in Amendment of 11/22/99)	
(4) in formal Fig. 16A:	5′-cca-ggt-ttg-aaa-gga-aaa-cgt-gga-...-3′
(5) in Sequence Listing (SEQ ID NO:9):	5′-cca-ggt-ttg-aaa-gga-aaa-cgt-gga-...-3′
U.S. App. No. 09/335,224: (as in Amendment filed 08/08/02)	
(6) substitute Fig. 16A:	5′-ggt-ttg-aaa-gga-aaa-cgt-gga-...-3′
(7) substitute Sequence Listing (SEQ ID NO:9)	5′-ggt-ttg-aaa-gga-aaa-cgt-gga-...-3′

As can be seen above, an extra codon ("CCA") was inadvertently introduced at the beginning of SEQ ID NO:9 in the present case during the preparation of the formal drawings and the Sequence Listing.

The substitute Fig. 18A and substitute Sequence Listing filed herewith correct SEQ ID NO:9 by removal of the nucleotides "CCA" at the beginning of the sequence, thereby returning this sequence to that depicted in Fig. 9 of U.S. App. No. 60/089,689.

Applicant has also found that SEQ ID NO:11, presented on page 108, line 15 of the present application, had an error introduced during the preparation of formal Fig. 18A. Specifically, the nucleotide "G" at position 9 of this sequence was incorrectly copied as "A" in

Fig. 18A. In addition, during the preparation of the present application, this sequence was replaced in the text as "CGG GAT CCA...". It is amended herein to "CGG GAT CCG...", as it was presented in U.S. App. No. 09/335,224. The depiction of SEQ ID NO:11 in the substitute formal Fig. 18A and the substitute Sequence Listing, both filed herewith, matches the original presentation of this sequence in the priority document.

Summary of Changes To Specification, Figs. 18A, 18B and Sequence Listing

The specification at page 44, lines 3-5 has been amended to state that Tumstatin 333 and 334 run from amino acids 1 to 124 and 125 to 244 of SEQ ID NO:10, respectively.

The specification at page 105, line 8 has been amended to present SEQ ID NO:11 as beginning with "CGG GAT CCG...", rather then "CGG GAT CCA...".

Exhibit B is a marked-up version of Fig. 18A as it was filed with the application on April 4, 2000. It shows that (1) SEQ ID NO:11 has been corrected from "CGG-GAT-CCA..." to "CGG-GAT-CCG...", (2) "CCA" has been removed from the beginning of SEQ ID NO:9, (3) the α3 chain has been corrected as running from nucleotides 1 through 732, (4) Tumstatin 333 has corrected as running from nucleotides 1 through 372, and (5) Tumstatin 334 has been corrected as running from nucleotides 373 through 732.

Exhibit C is marked-up version of formal Fig. 18B as it was filed with the application on April 4, 2000, and shows that (1) the amino acid residue "P" has been removed from the beginning of SEQ ID NO:10, (2) the length of the protein is now 244 amino acids, (3) the α3 chain has been corrected as running from residues 1 through 244, (4) Tumstatin 333 has been corrected as running from residues 1 through 124, and (5) Tumstatin 334 has been corrected as running from residues 125 through 244.

Exhibit D is a copy of pages 5-8 of the Sequence Listing as it was filed February 8, 2001, marked up to show the corrections made in the Sequence Listing filed herewith. It shows that in SEQ ID NO:9, (1) the first three nucleotides ("CCA") have been removed, (2) the coding sequence (CDS) has been corrected to run from nucleotides 1 to 732, (3) Tumstatin N53 has been corrected to run from nucleotides 160 to 732, (4) Tumstatin 333 has been corrected to run from nucleotides 1 to 372, (5) Tumstatin 334 has been corrected to run from nucleotides 373 to

732, and (6) SEQ ID NO:10, which was correctly presented as being 244 amino acids long in the Sequence Listing of February 8, 2001, is presented the same way in the substitute Sequence Listing filed herewith.

Applicants submit that in view of the foregoing remarks, all issues relevant to sequence compliance have been addressed, and request that the objection on this basis be withdrawn.

Information Disclosure Statement

The Examiner states that the Information Disclosure Statement filed January 4, 2002 has not been considered because it failed to comply with 37 C.F.R. § 1.97(c) because it lacked the fee under 37 C.F.R. § 1.17(p) and the statement under 37 C.F.R.§ 1.97(e). Applicants respectfully submit that this is not true.

37 C.F.R. 1.97(c) states that:

> An information disclosure statement **shall be** considered by the Office if filed after the period specified in paragraph (b) of this section, provided that the information disclosure statement is filed before the mailing date of any of a final action under § 1.113, and notice of allowance under § 1.311, or an action that otherwise closes prosecution in the application, and it is accompanied by one of:
>
> (1) The statement specified in paragraph (e) of this section; **or**
>
> (2) The fee set forth in § 1.17(p).

(emphasis added).

Applicants note that in the Information Disclosure Statement filed on January 4, 2002, the Office had been authorized to charge any necessary fees to a particular Deposit Account. Applicants respectfully submit that any required fees should have been taken from the Deposit Account according to 37 C.F.R. § 1.25(b), which states that:

> (b) Filing, issue, appeal, international-type search report, international application processing, petition, and post-issuance fees may be charged against these accounts if sufficient funds are on deposit to cover such fees. A general authorization to charge all fees, or only certain fees, set forth in §§ 1.16 to 1.18 to a deposit account containing sufficient funds may be filed in an individual application, either for the entire pendency of the application **or with a particular paper filed**.

The Information Disclosure Statement filed on January 4, 2002 should therefore have been considered by the Office, and not simply "placed in the application file." Applicant also notes that in section 17 of the present Office Action, the Examiner has accepted Applicant's provision of two related applications that were listed in the Information Disclosure Statement, but has simply chosen not to consider the other references cited therein.

Applicants re-submit the Information Disclosure Statement herewith, with the statement under 37 C.F.R. § 1.97(e) that as of January 4, 2002, all of the items listed on the form PTO-1449 submitted on January 4, 2002 were listed on the the International Search Report for related application PCT/US01/00565, which, as of January 4, 2002, was mailed not more than three months prior to the January 4, 2002 filing of the Information Disclosure Statement. The fee under 37 C.F.R. §1.17(p) is also enclosed herewith.

Applicants are therefore re-submitting the Information Disclosure Statement of January 4, 2002 under37 C.F.R. § 1.(d), which requires both the fee and the statement. Applicants respectfully submit that the requirements of 37 C.F.R. § 1.97 have been complied with, and request that the Information Disclosure Statement and the references cited therein be considered.

Title of Application

Applicants have amended the title to: "Anti-Angiogenic alpha-v-beta-3 Integrin-Binding Collagen Peptides and Methods of Use Thereof". Acceptance of the amendment to the title and withdrawal of the rejection on this basis are respectfully requested.

Abstract

Applicant has amended the Abstract. Acceptance of the new Abstract and withdrawal of the rejection on this basis are respectfully requested.

Priority Claims

The Examiner has stated that the priority date of claims 1-3 is June 17, 1999, the filing date of U.S. App. No. 09/335,224, and that the priority date of claims 4 and 8-9 is April 4, 2000, the date of the present application.

Claims 8 is amended herein to recite an isolated fragment having the sequence of amino acids 1 to 124 of SEQ ID NO:10. As claim 8 as presently amended effectively recites the sequence of Tumstatin 333, which is presented in U.S. App. No. 09/335,224, Applicant respectfully submits that the priority date of claim 8 is at least as early as June 17, 1999.

In the Reply to the Office Action of June 5, 2001, Applicant noted that the Examiner has not met the initial burden (as set out in the "Guidelines for Examination of Patent Applications Under the 35 U.S.C. § 112, ¶1, 'Written Description' Requirement" (*Federal Register*, Vol. 66, No. 4, January 5, 2001) of presenting evidence or reasoning as to why persons skilled in the art would not recognize in the original disclosure a description of the invention defined by the claims, that is, fragments of the sequences described, especially given that the priority documents discuss that fragments of the full-length proteins can have the same properties as their parent molecules, and given that exactly such fragments were presented in U.S. App. No. 09/335,224. Methods of subdividing protein sequences and of testing the resulting peptides are well-known in the art, and Applicant used precisely such well-known methods to produce the fragments now claimed. The disclosures of the prior application would therefore "reasonably convey" (as required in *Ex parte Sorenson*, 3.U.S.P.Q.2d 1462 (Bd. Pat. App. Interf. 1987)) to one of ordinary skill in the art that the properties described could be found in fragments of the full-length proteins.

The Examiner has concluded, however, that the disclosure of a genus of anti-angiogenic fragments in the priority documents does not provide support for the species of precise fragments disclosed in later applications, and relies on several cases, including *Purdue Pharma L.P. v. Faulding Inc.*, 230 F.3d 1320, 56 U.S.P.Q.2d 1481 (Fed. Cir. 2000); *In re Ruschig*, 379 F.2d 990, 154 U.S.P.Q. 118 (C.C.P.A. 1967); *Fujikawa v. Wattanasin*, 93 F.3d 1559, 39 U.S.P.Q.2d 1895 (Fed. Cir. 1996); *Martin v. Mayer*, 823 F.2d 500, 3 U.S.P.Q.2d 1333 (Fed. Cir. 1987); *Jepson v. Coleman*, 314 F.2d 533, 136 U.S.P.Q. 647 (C.C.P.A. 1963).

The Court in *Purdue Pharma* discussed *Ruschig*, noting that the specification at issue in *Ruschig* disclosed a half a million compounds, with no guidance leading one to a particular claimed compound. The Court in *Ruschig* addressed the issue of whether a claim to the specific compound N-(p-chlorobenzenesulfonyl)-N'-propylurea was supported by a disclosure which

listed reagents for the preparation of chlorpropamide, where, if one happened to choose, for each of three different required chemicals, one specific compound from the various alternative chemicals presented, the synthesis would *just happen* to produce the claimed compound N-(p-chlorobenzenesulfonyl)-N'-propylurea ("If the proper choices of the three variables . . . are made, the compound in question is produced" (at 121)).

The situation in *Ruschig* is not in any way analogous to the present application. In *Ruschig*, the majority of the "choices" in starting materials for synthesis of the claimed compound would not even have resulted in that compound. In contrast, in the present application and the priority documents, Applicant clearly set forth three proteins of finite length (Arresten, Canstatin, Tumstatin), where the proteins possessed a specific, readily-assayable property (anti-angiogenicity). Applicant also disclosed in the present application and the priority documents that claimed fragments of these proteins that also possessed the same property were to be considered part of the invention. Unlike *Ruschig*, where there were no specific pointers to the claimed compound, Applicant clearly indicated that a number of the further subdivisionas of each of the anti-angiogenic proteins were likely, expected even, to possess the same activity. All that was required was that the inactive portions be carved away.

Fujikawa was an interference involving two counts, one to a genus of mevalolactones, and one to a method of using same to inhibit cholesterol biosynthesis. Fujikawa, the senior party, appealed both the award of priority to Wattanasin, and also the denial of the addition of a sub-genus count. Being an interference, Fujikawa was required to find the support for such a sub-genus in the disclosure of Wattanasin. Both parties agreed that the *individual* constituents recited in the proposed sub-genus count were adequately represented in Wattanasin's application, however, the Board held that there was insufficient indication that would lead one to assemble the individual consituents into the proposed sub-genus. In other words, there was insufficient disclosure to indicate to one of ordinary skill that a sub-genus of particular compounds was to be differentiated from the overall genus.

Like *Ruschig*, the situation addressed in *Fujikawa* is not applicable here. Applicant has always maintained that one of ordinary skill could easily isolate anti-angiogenic fragments of the overall proteins according to the guidance already provided in the priority applications. No

additional guidance or characteristics are needed to isolate new anti-angiogenic fragments of the overall proteins. Both *Fujikawa* and *Ruschig* are therefore inapposite.

Martin is another case involving an interference. Martin, the junior party, appealed the issue of whether Mayer (whose specification disclosed a cable of a particular construction) could present a count to a harness composed of a plurality of the cables without pointing to the support for same. Like the above cases, Applicant's disclosure is not analogous to the facts of *Martin*. *Martin* involved whether disclosure of a cable supported claims to a device (a harness) made out of the cables. Applicant's disclosure, in contrast, is to particular full-length proteins with a defined activity, and fragments of those proteins also possessing the activity.

The present application is easily distinguished from these cases in that Applicant has disclosed specific proteins with an unusual property, noting that the same property was likely to reside in subdivisions of the proteins. Applicant produced exactly such fragments which did indeed possess the property, using the methods disclosed in the specification. Furthermore, one of ordinary skill in the art (or a potential infringer) could also have easily done so.

Applicant further notes that U.S. App. No. 09/335,224 has an identical counterpart International Application that was published as WO 99/65940. One of ordinary skill, upon reading that disclosure, would have all of the information needed to isolate and assay additional anti-angiogenic fragments, including those disclosed in the present application. Under the Examiner's reasoning, anyone can use the teachings of WO 99/65940 to isolate anti-angiogenic fragments, while Applicant would be prevented from protecting the fruits of his discovery and would be afforded only later priority dates for those same fragments. The Examiner's conclusions effectively gives free rein to copyists, and would discourage applicants from allowing publication of their applications, and encourage protection by secrecy, rather than patent.

Applicant therefore respectfully requests that the refusal of priority on these grounds be reconsidered and withdrawn.

Objection to the Specification and Antecedent Basis

The Examiner has objected to the specification as failing to provide antecedent basis for claim 8.

Claim 8 has been amended, obviating the objection.

Objection to the Specification Regarding Hyperlinks

The Examiner has objected to the specification because it includes embedded hyperlinks.

The specification has been amended, obviating the objection.

Applicants respectfully request entry of the above amendments.

Date: September 11, 2002

Respectfully submitted,

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MARKED-UP VERSION OF AMENDMENTS:

Specification Amendments Under 37 C.F.R. § 1.121(b)(1)(iii)

Please replace the Title with the Title as shown below, which is marked up by way of bracketing and underlining to show the changes relative to the previous version of the Title:

Anti-Angiogenic alpha-v-beta-3 Integrin-Binding Collagen Peptides [Proteins and Fragments] and Methods of Use Thereof

Please replace the Abstract with the Abstract as shown below, which is marked up by way of bracketing and underlining to show the changes relative to the previous version of the Abstract:

Anti-angiogenic proteins and peptides isolated from the non-Goodpasture region of α3(IV) NC1 domain of collagen are disclosed, which have the ability to bind $\alpha_v\beta_3$ integrin, and/or inhibit proliferation of endothelial cells. [Proteins with anti-angiogenic properties are disclosed, and fragments thereof, and methods of using those proteins and fragments to inhibit or promote angiogenisis.]

Please replace the paragraph at page 20, lines 17 through 22, with the paragraph below, which is marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph:

Figs. 24A, 24B, 24C and 24D are a set of four histograms [fourhistograms] showing binding of HUVEC cells to plates coated

with Tumstatin (Fig. 24A), or controls of type IV collagen (Fig. 24B), vitronectin (Fig. 24C) or laminin-1 (Fig. 24A) in the presence of integrin subunits α_1 through α_6, β_1, or $\alpha_V\beta_3$ integrin blocking antibody. The plate coating is listed at the top of each graph, and the antibodies used for incubation are on the x-axis of each graph. BSA-coated plates were used as negative controls.

Please replace the paragraph at page 43, line 23 through page 44, line 5, with the paragraph below, which is marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph:

One such fragment, designated "Tumstatin N-53", was found to have anti-angiogenic activity equivalent to that of full-length Tumstatin, as determined by standard assays. Tumstatin N-53 comprises a Tumstatin molecule wherein the N-terminal 53 amino acids have been deleted. Other mutant fragments described herein have been found to have very high levels of anti-angiogenic activity, as shown by the assays described herein. These fragments, "Tumstatin 333," "Tumstatin 334," "12 kDa Arresten fragment," "8 kDa Arresten fragment," and "10 kDa Canstatin fragment" have ED_{50} values of 75 ng/ml, 20 ng/ml, 50 ng/ml, 50 ng/ml, and 80 ng/ml, respectively. By contrast, full-length Arresten, Canstatin and Tumstatin were found to have ED_{50} values of 400 ng/ml, 400 ng/ml, and 550 ng/ml, respectively. Tumstatin 333 comprises amino acids 1[2] to 124[125] of SEQ ID NO:10, and Tumstatin 334 comprises amino acids 125[126] to 244[245] of SEQ ID NO:10.

Please replace the paragraph at page 47, lines 20 through 26 with the paragraph below, which is marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph:

> Identity is often measured using sequence analysis software *e.g.*, BLASTN or BLASTP (available at the world wide web site ("www") for the National Center for Biotechnology Information (".ncbi") of the National Institutes of Health (".nih") of the U.S. government (".gov"), in the "/BLAST/" directory [http://www.ncbi.nlm.nih.gov/BLAST/]). The default parameters for comparing two sequences (*e.g.*, "Blast"-ing two sequences against each other[, http://www.ncbi.nlm.nih.gov/gorf/bl2.html]) by BLASTN (for nucleotide sequences) are reward for match = 1, penalty for mismatch = -2, open gap = 5, extension gap = 2. When using BLASTP for protein sequences, the default parameters are reward for match = 0, penalty for mismatch = 0, open gap = 11, and extension gap = 1.

Please replace the paragraph at page 105, line 2 through page 106, line 7 with the paragraph below, which is marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph:

> The nucleotide (SEQ ID NO:9) and amino acid (SEQ ID NO:10) sequences for the α3 chain of the NC1 domain of Type IV collagen are shown in Figs. 18A and 18B, respectively. The sequence encoding Tumstatin was amplified by PCR from the α3 NCI (IV)/pDS vector (Neilson, E.G. *et al.*, 1993, *J. Biol. Chem.* 268:8402-5; GenBank Accession Nos. M92993 (Quinones, S. *et al.*, 1994), M81379 (Turner, N. *et al.*, 1994), and X80031 (Leionin,

A.K., and Mariyama, M. *et al.*, 1998)) using the forward primer 5'-CGG GAT CCG [CCA] GGT TTG AAA GGA AAA CGT-3' (SEQ ID NO:11) and the reverse primer 5'- CCC AAG CTT TCA GTG TCT TTT CTT CAT-3' (SEQ ID NO:12). The resulting cDNA fragment was digested with *Bam*HI and *Hin*dIII and ligated into predigested pET22b(+) (Novagen, Madison, Wisconsin, USA). The construct is shown in Fig. 19. The ligation placed Tumstatin downstream of and in-frame with the pelB leader sequence, allowing for periplasmic localization and expression of soluble protein. Additional vector sequence was added to the protein encoding amino acids MDIGINSD (SEQ ID NO:13). The 3' end of the sequence was ligated in-frame with the polyhistidine tag sequence. Additional vector sequence between the 3' end of the cDNA and the his-tag encoded the amino acids KLAAALE (SEQ ID NO:14). Positive clones were sequenced on both strands. Plasmid constructs encoding Tumstatin were first transformed into *E. coli* HMS174 (Novagen, Madison, Wisconsin, USA) and then transformed into BL21 for expression (Novagen, Madison, Wisconsin, USA). Overnight bacterial culture was used to inoculate a 500 ml culture in LB medium (Fisher Scientific, Pittsburgh, Pennsylvania, USA). This culture was grown for approximately 4 hours until the cells reached an OD_{600} of 0.6. Protein expression was then induced by addition of IPTG to a final concentration of 1 mM. After a 2-hour induction, cells were harvested by centrifugation at 5,000 x g and lysed by resuspension in 6 M guanidine, 0.1 M NaH_2PO_4, 0.01 M Tris-HCl, pH 8.0. Resuspended cells were sonicated briefly, and centrifuged at 12,000 x g for 30 minutes. The supernatant fraction was passed over a 5 ml Ni-NTA agarose column (Qiagen, Hilden, Germany)

4-6 times at a speed of 2 ml per minute. Non-specifically bound protein was removed by washing with both 10 mM and 25 mM imidazole in 8 M urea, 0.1 M NaH_2PO_4, 0.01 M Tris-HCl, pH 8.0. Tumstatin protein was eluted from the column with increasing concentrations of imidazole (50 mM, 125 mM, and 250 mM) in 8 M urea, 0.1 M NaH_2PO_4, 0.01 M Tris-HCl, pH 8.0. The eluted protein was dialyzed twice against PBS at 4°C. A portion of the total protein precipitated during dialysis. Dialyzed protein was collected and centrifuged at approximately 3,500 x g and separated into insoluble (pellet) and soluble (supernatant) fractions.

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

Please amend claims 4 and 9 as follows:

4. (Twice Amended) An isolated fragment of α3(IV) NC1 domain, having the amino acid sequence of amino acid residue 53 [54] to amino acid 123 [124] of SEQ ID NO:10.

9. (Amended) An isolated fragment of α3(IV) NC1 domain, having the amino acid sequence of amino acid residue 180 [181] to amino acid residue 245 [244] of SEQ ID NO:10.

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Fig. 9

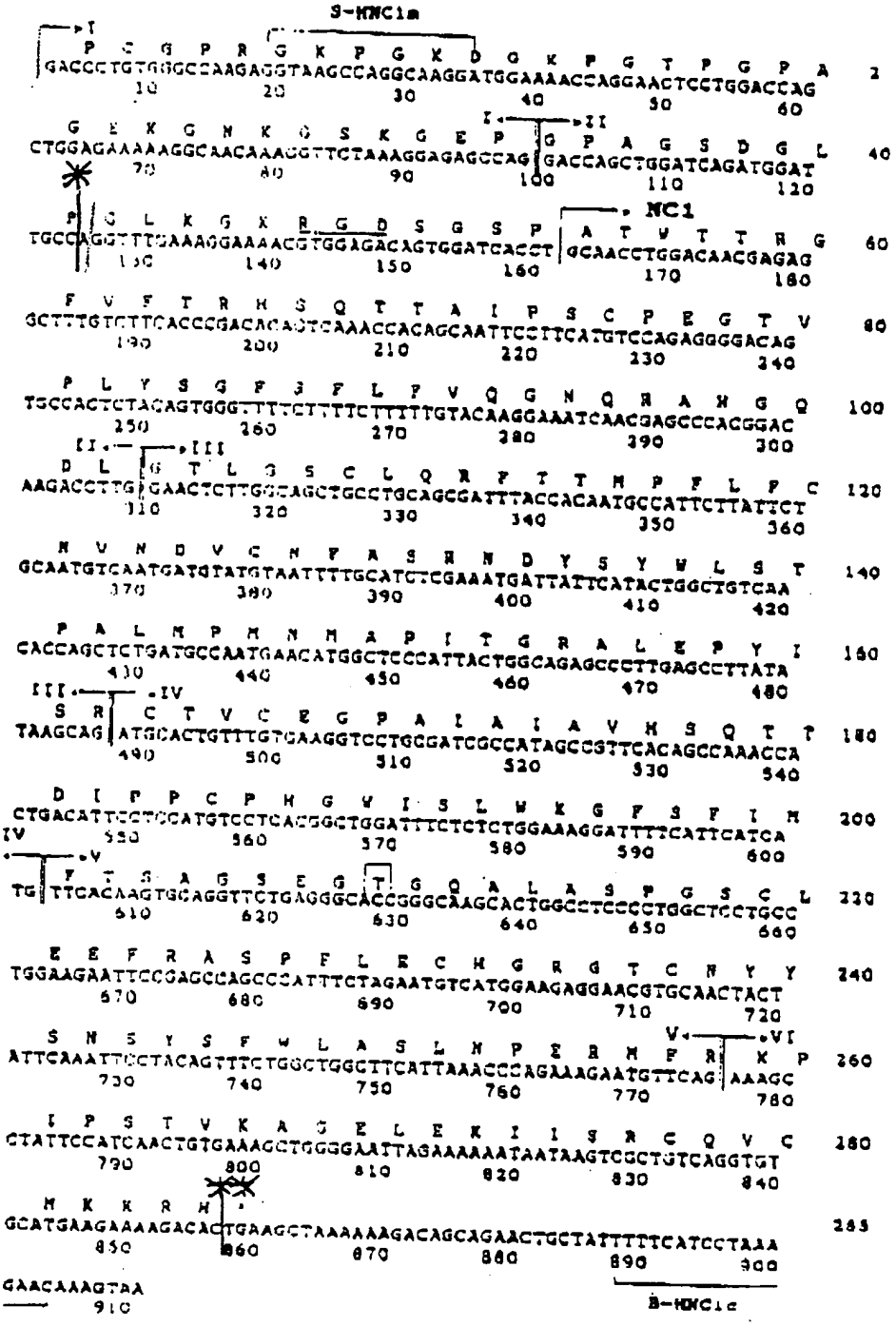

FIG. 2. Nucleotide and derived amino acid sequence of HGA3.3 exons. *Bent arrows* indicate the 5'- and 3'-borders of each exon and the beginning of the NC1 domain. The RGD sequence is *underlined*. The *boxed* amino acid is different from that previously reported (11). Positions of the oligonucleotides S-HNC1m and B-HNC1c used for PCR amplification of a human α3(IV) cDNA are indicated. Amino Acids 1–67 have not been previously reported (11).

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          10          20          30          40
     *    *    *    *    *    *    *    *    *
GLK GKR GDS GSP ATW TTR GFV FTR HSQ TTA IPS CPE GTV PLY SGF
_____1427 TO 1670 OF FULL LENGTH A3 CHAIN OF TYPE IV_____>

          50          60          70          80          90
     *    *    *    *    *    *    *    *    *    *
SFL FVQ GNQ RAH GQD LGT LGS CLQ RFT TMP FLP CNV NDV CNF ASR
_____1427 TO 1670 OF FULL LENGTH A3 CHAIN OF TYPE IV_____>

          100         110         120         130
     *    *    *    *    *    *    *    *    *
NDY SYW LST PAL MPM NMA PIT GRA LEP YIS RCT VCE GPA IAI AVH
_____1427 TO 1670 OF FULL LENGTH A3 CHAIN OF TYPE IV_____>

   140         150         160         170         180
     *    *    *    *    *    *    *    *    *
SQT TDI PPC PHG WIS LWK GFS FIM FTS AGS EGT GQA LAS PGS CLE
_____1427 TO 1670 OF FULL LENGTH A3 CHAIN OF TYPE IV_____>

          190         200         210         220
     *    *    *    *    *    *    *    *    *
EFR ASP FLE CHG RGT CNY YSN SYS FWL ASL NPE RMF RKP IPS TVK
_____1427 TO 1670 OF FULL LENGTH A3 CHAIN OF TYPE IV_____>

   230         240
     *    *    *
AGE LEK IIS RCQ VCM KKR H
___1427 TO 1670 OF FU____>
```

Fig. 10

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FIG. 18A

pET22b(+) forward primer:
5'-CGGGAT CCA GGT TTG AAA GGA AAA CGT-3' (SEQ ID NO:11)
pET22b(+) reverse primer:
5'-CCCAAGCTT TCA GTG TCT TTT CTT CAT-3' (SEQ ID NO:12)

```
    5     10    15    20    25    30    35    40    45
caa ggt ttg aaa gga aaa cgt gga gac agt gga tca cct gca acc
   50    55    60    65    70    75    80    85    90
tgg aca acg aga ggc ttt gtc ttc acc cga cac agt caa acc aca
   95   100   105   110   115   120   125   130   135
gca att cct tca tgt cca gag ggg aca gtg cca ctc tac agt ggg
  140   145   150   155   160   165   170   175   180
ttt tct ttt ctt ttt gta caa gga aat caa cga gcc cac gga caa
  185   190   195   200   205   210   215   220   225
gac ctt gga act ctt ggc agc tgc ctg cag cga ttt acc aca atg
  230   235   240   245   250   255   260   265   270
cca ttc tta ttc tgc aat gtc aat gat gta tgt aat ttt gca tct
  275   280   285   290   295   300   305   310   315
cga aat gat tat tca tac tgg ctg tca aca cca gct ctg atg cca
  320   325   330   335   340   345   350   355   360
atg aac atg gct ccc att act ggc aga gcc ctt gag cct tat ata
  365   370   375   380   385   390   395   400   405
agc aga tgc act gtt tgt gaa ggt cct gcg atc gcc ata gcc gtt
  410   415   420   425   430   435   440   445   450
cac agc caa acc act gac att cct cca tgt cct cac ggc tgg att
  455   460   465   470   475   480   485   490   495
tct ctc tgg aaa gga ttt tca ttc atc atg ttc aca agt gca ggt
  500   505   510   515   520   525   530   535   540
tct gag ggc acc ggg caa gca ctg gcc tcc cct ggc tcc tgc ctg
  545   550   555   560   565   570   575   580   585
gaa gaa ttc cga gcc agc cca ttt cta gaa tgt cat gga aga gga
  590   595   600   605   610   615   620   625   630
acg tgc aac tac tat tca aat tcc tac agt ttc tgg ctg gct tca
  635   640   645   650   655   660   665   670   675
tta aac cca gaa aga atg ttc aga aag cct att cca tca act gtg
  680   685   690   695   700   705   710   715   720
aaa gct ggg gaa tta gaa aaa ata ata agt cgc tgt cag gtg tgc
  725   730   735
atg aag aaa aga cac tga        (SEQ ID NO:9)
```

pET22b-α3(IV) NC1 = nucleotides 1 through 735 732
Tumstatin 333 = nucleotides 1 through 375 372
Tumstatin 334 - nucleotide 376 373 through 735 732

Exhibit B

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FIG. 18B

```
*
     5     10    15     20     25     30     35     40     45
PGL KGK RGD SGS PAT WTT RGF VFT RHS QTT AIP SCP EGT VPL YSG

    50     55    60     65     70     75     80     85     90
FSF LFV QGN QRA HGQ DLG TLG SCL QRF TTM PFL FCN VND VCN FAS

                                          *+
    95    100   105    110    115    120    125    130    135
RND YSY WLS TPA LMP MNM API TGR ALE PYI SRC TVC EGP AIA IAV

   140    145   150    155    160    165    170    175    180
HSQ TTD IPP CPH GWI SLW KGF SFI MFT SAG SEG TGQ ALA SPG SCL

   185    190   195    200    205    210    215    220    225
EEF RAS PFL ECH GRG TCN YYS NSY SFW LAS LNP ERM FRK PIP STV

                +
   230    235   240   245 244
KAG ELE KII SRC QVC MKK RH      (SEQ ID NO:10)
```

pET22b α3(IV) NC1 = residues 1 through 245 244
Tumstatin 333 = residues 1 through 125 124
Tumstatin 334 = residues 126 through 245
125 244

Exhibit C

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<212> PRT
<213> Homo sapiens

<400> 6
Val Ser Ile Gly Tyr Leu Leu Val Lys His Ser Gln Thr Asp Gln Glu
1 5 10 15
Pro Met Cys Pro Val Gly Met Asn Lys Leu Trp Ser Gly Tyr Ser Leu
20 25 30
Leu Tyr Phe Glu Gly Gln Glu Lys Ala His Asn Gln Asp Leu Gly Leu
35 40 45
Ala Gly Ser Cys Leu Ala Arg Phe Ser Thr Met Pro Phe Leu Tyr Cys
50 55 60
Asn Pro Gly Asp Val Cys Tyr Tyr Ala Ser Arg Asn Asp Lys Ser Tyr
65 70 75 80
Trp Leu Ser Thr Thr Ala Pro Leu Pro Met Met Pro Val Ala Glu Asp
85 90 95
Glu Ile Lys Pro Tyr Ile Ser Arg Cys Ser Val Cys Glu Ala Pro Ala
100 105 110
Ile Ala Ile Ala Val His Ser Gln Asp Val Ser Ile Pro His Cys Pro
115 120 125
Ala Gly Trp Arg Ser Leu Trp Ile Gly Tyr Ser Phe Leu Met His Thr
130 135 140
Ala Ala Gly Asp Glu Gly Gly Gly Gln Ser Leu Val Ser Pro Gly Ser
145 150 155 160
Cys Leu Glu Asp Phe Arg Ala Thr Pro Phe Ile Glu Cys Asn Gly Gly
165 170 175
Arg Gly Thr Cys His Tyr Tyr Ala Asn Lys Tyr Ser Phe Trp Leu Thr
180 185 190
Thr Ile Pro Glu Gln Ser Phe Gln Gly Ser Pro Ser Ala Asp Thr Leu
195 200 205
Lys Ala Gly Leu Ile Arg Thr His Ile Ser Arg Cys Gln Val Cys Met
210 215 220
Lys Asn Leu
225

<210> 7
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> pET22b(+) forward oligonucleotide primer for Canstatin

<400> 7
cgggatcctg tcagcatcgg ctacctc 27

<210> 8
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> pET22b(+) reverse oligonucleotide primer for Canstatin

<400> 8
cccaagcttc aggttcttca tgcacac 27

<210> 9

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<211> ~~738~~ 735
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> ~~(4)~~...~~(735)~~ 732

<221> misc_feature
<222> (160)...~~(735)~~ 732
<223> Tumstatin N53

<221> misc_feature
<222> ~~(4)~~...~~(375)~~ 1-372
<223> Tumstatin 333

<221> misc_feature
<222> ~~(376)~~..~~(735)~~ 373-732
<223> Tumstatin 334

<400> 9

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cca ggt ttg aaa gga aaa cgt gga gac agt gga tca cct gca acc tgg       48
    Gly Leu Lys Gly Lys Arg Gly Asp Ser Gly Ser Pro Ala Thr Trp
     1               5                  10                  15

aca acg aga ggc ttt gtc ttc acc cga cac agt caa acc aca gca att       96
Thr Thr Arg Gly Phe Val Phe Thr Arg His Ser Gln Thr Thr Ala Ile
                20                  25                  30

cct tca tgt cca gag ggg aca gtg cca ctc tac agt ggg ttt tct ttt      144
Pro Ser Cys Pro Glu Gly Thr Val Pro Leu Tyr Ser Gly Phe Ser Phe
            35                  40                  45

ctt ttt gta caa gga aat caa cga gcc cac gga caa gac ctt gga act      192
Leu Phe Val Gln Gly Asn Gln Arg Ala His Gly Gln Asp Leu Gly Thr
        50                  55                  60

ctt ggc agc tgc ctg cag cga ttt acc aca atg cca ttc tta ttc tgc      240
Leu Gly Ser Cys Leu Gln Arg Phe Thr Thr Met Pro Phe Leu Phe Cys
    65                  70                  75

aat gtc aat gat gta tgt aat ttt gca tct cga aat gat tat tca tac      288
Asn Val Asn Asp Val Cys Asn Phe Ala Ser Arg Asn Asp Tyr Ser Tyr
80                  85                  90                  95

tgg ctg tca aca cca gct ctg atg cca atg aac atg gct ccc att act      336
Trp Leu Ser Thr Pro Ala Leu Met Pro Met Asn Met Ala Pro Ile Thr
                100                 105                 110

ggc aga gcc ctt gag cct tat ata agc aga tgc act gtt tgt gaa ggt      384
Gly Arg Ala Leu Glu Pro Tyr Ile Ser Arg Cys Thr Val Cys Glu Gly
            115                 120                 125

cct gcg atc gcc ata gcc gtt cac agc caa acc act gac att cct cca      432
Pro Ala Ile Ala Ile Ala Val His Ser Gln Thr Thr Asp Ile Pro Pro
        130                 135                 140
```

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```
tgc cct cac ggc tgg att tct ctc tgg aaa gga ttt tca ttc atc atg      480
Cys Pro His Gly Trp Ile Ser Leu Trp Lys Gly Phe Ser Phe Ile Met
    145                 150                 155

ttc aca agt gca ggt tct gag ggc acc ggg caa gca ctg gcc tcc cct      528
Phe Thr Ser Ala Gly Ser Glu Gly Thr Gly Gln Ala Leu Ala Ser Pro
160                 165                 170                 175

ggc tcc tgc ctg gaa gaa ttc cga gcc agc cca ttt cta gaa tgt cat      576
Gly Ser Cys Leu Glu Glu Phe Arg Ala Ser Pro Phe Leu Glu Cys His
                180                 185                 190

gga aga gga acg tgc aac tac tat tca aat tcc tac agt ttc tgg ctg      624
Gly Arg Gly Thr Cys Asn Tyr Tyr Ser Asn Ser Tyr Ser Phe Trp Leu
            195                 200                 205

gct tca tta aac cca gaa aga atg ttc aga aag cct att cca tca act      672
Ala Ser Leu Asn Pro Glu Arg Met Phe Arg Lys Pro Ile Pro Ser Thr
        210                 215                 220

gtg aaa gct ggg gaa tta gaa aaa ata ata agt cgc tgt cag gtg tgc      720
Val Lys Ala Gly Glu Leu Glu Lys Ile Ile Ser Arg Cys Gln Val Cys
    225                 230                 235

atg aag aaa aga cac tga                                             738
Met Lys Lys Arg His
240
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<210> 10
<211> 244
<212> PRT
<213> Homo sapiens

<220>
<221> PEPTIDE
<222> (54)...(245)
<223> Tumstatin N53

<221> PEPTIDE
<222> ~~(2)~~...~~(125)~~ 1 - 124
<223> Tumstatin 333

<221> PEPTIDE
<222> ~~(126)~~...~~(245)~~ 125-244
<223> Tumstatin 334

<400> 10

```
Gly Leu Lys Gly Lys Arg Gly Asp Ser Gly Ser Pro Ala Thr Trp Thr
 1               5                  10                  15
Thr Arg Gly Phe Val Phe Thr Arg His Ser Gln Thr Thr Ala Ile Pro
            20                  25                  30
Ser Cys Pro Glu Gly Thr Val Pro Leu Tyr Ser Gly Phe Ser Phe Leu
        35                  40                  45
Phe Val Gln Gly Asn Gln Arg Ala His Gly Gln Asp Leu Gly Thr Leu
    50                  55                  60
Gly Ser Cys Leu Gln Arg Phe Thr Thr Met Pro Phe Leu Phe Cys Asn
65                  70                  75                  80
```

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```
Val Asn Asp Val Cys Asn Phe Ala Ser Arg Asn Asp Tyr Ser Tyr Trp
                85                  90                  95
Leu Ser Thr Pro Ala Leu Met Pro Met Asn Met Ala Pro Ile Thr Gly
            100                 105                 110
Arg Ala Leu Glu Pro Tyr Ile Ser Arg Cys Thr Val Cys Glu Gly Pro
        115                 120                 125
Ala Ile Ala Ile Ala Val His Ser Gln Thr Thr Asp Ile Pro Pro Cys
    130                 135                 140
Pro His Gly Trp Ile Ser Leu Trp Lys Gly Phe Ser Phe Ile Met Phe
145                 150                 155                 160
Thr Ser Ala Gly Ser Glu Gly Thr Gly Gln Ala Leu Ala Ser Pro Gly
                165                 170                 175
Ser Cys Leu Glu Glu Phe Arg Ala Ser Pro Phe Leu Glu Cys His Gly
            180                 185                 190
Arg Gly Thr Cys Asn Tyr Tyr Ser Asn Ser Tyr Ser Phe Trp Leu Ala
        195                 200                 205
Ser Leu Asn Pro Glu Arg Met Phe Arg Lys Pro Ile Pro Ser Thr Val
    210                 215                 220
Lys Ala Gly Glu Leu Glu Lys Ile Ile Ser Arg Cys Gln Val Cys Met
225                 230                 235                 240
Lys Lys Arg His
```

<210> 11
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> pET22b(+) forward oligonucleotide primer for Tumstatin

<400> 11
cgggatccgg gtttgaaagg aaaacgt 27

<210> 12
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> pET22b(+) reverse oligonucleotide primer for Tumstatin

<400> 12
cccaagcttt cagtgtcttt tcttcat 27

<210> 13
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Additional vector sequence added to protein

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Met Asp Ile Gly Ile Asn Ser Asp
 1               5
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<210> 14
<211> 7